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EXAMINER
NGUYEN, QUANG

ART UNIT	PAPER NUMBER
1636	9

DATE MAILED: 06/05/2002

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application N .	Applicant(s)	
	09/865,022	HEBBEL ET AL.	
	Examiner	Art Unit	
	Quang Nguyen, Ph.D	1636	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *18 April 2002* .

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-44* is/are pending in the application.

4a) Of the above claim(s) *15-43* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-14 and 44* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

11)☐ The proposed drawing correction filed on _____ is: a)☐ approved b)☐ disapproved by the Examiner.

If approved, corrected drawings are required in reply to this Office action.

12)☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. §§ 119 and 120

13)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____ .

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

14)☒ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. § 119(e) (to a provisional application).

a) ☐ The translation of the foreign language provisional application has been received.

15)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. §§ 120 and/or 121.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO-1449) Paper No(s) 5 .
4) ☐ Interview Summary (PTO-413) Paper No(s). _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: .

DETAILED ACTION

Applicants' amendment filed April 18, 2002 in Paper No. 8 has been entered.

Claims 1-44 are pending in the present application.

Applicants' election with traverse the invention of Group I (Claims 1-14) in the Supplementary Preliminary Amendment and Response to Restriction Requirement in Paper No. 8 is acknowledged.

Applicants argued that the inventions are so closely related within the context of the disclosure of the application that they cannot properly be considered independent and distinct within the statutory meaning of 35 U.S.C. 121. Applicants further argued that the search and examination of an entire application can be made in a single search without any undue burden for the Examiner. Applicants' arguments are respectfully found to be unpersuasive because the inventions are distinct for the reasons already set forth in Paper No. 7, and that they have acquired a separate status in the art because of their recognized divergent subject matter, and separate search requirements. Therefore, it would be unduly burdensome for the Examiner to search and/or consider the patentability of all the inventions in a single application. Moreover, Applicants are entitled to a single distinct invention per application. With respect to the newly added claims 37-44, claim 44 drawn to a population of expanded endothelial cells prepared by the method of claim 1 is grouped together with claims 1-14 of Group I, whereas claims 37-44 drawn to a transgenic endothelial cell comprising a vector comprising an isolated DNA sequence encoding a preselected proteins operably linked to a promoter functional in human endothelial cells, which transgenic endothelial cell is prepared by introducing

the vector into an expanded population of endothelial cells prepared by culturing, in contact with a collagen I-coated surface, buffy coat cells obtained from peripheral mammalian blood in the presence of a cell culture medium containing an effective amount of vascular endothelial growth factor (VEGF) and which medium is free of bovine brain extract are grouped together with claims 15-26 of Group II. This is because a transgenic endothelial cell of claims 37-43 is prepared by a process that has distinct method steps and starting materials from the process of claim 1. For example, the process of claim 1 is merely for expanding a population of endothelial cells obtained from peripheral blood without requiring any transfection or transfomation of the expanded endothelial cells with a vector comprising a DNA sequence encoded a preselected protein as needed by a process to make the transgeneic endothelial cell. Furthermore, by expanding a population of endothelial cells the method of claim 1 is already complete. Therefore, restriction for examination purposes as indicated is proper and this is made FINAL.

Accordingly, claims 15-43 are withdrawn from further consideration because they are drawn to non-elected inventions.

Claims 1-14 and newly added claim 44 are examined on the merits herein.

Claim Rejections - 35 USC § 112

The following is a quotation of the second paragraph of 35 U.S.C. 112:

The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims 1-14 and 44 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claim 1 and its dependent claims recite the limitation "the population of endothelial cells obtained from peripheral blood" in lines 1 and 2 of the claim. There is insufficient antecedent basis for this limitation in the claim. It is unclear which cell populations of endothelial cells are obtained from peripheral blood? Addtionally, the phrase "expand the populatation of endothelial cells in said buffy coat cells" is unclear. How can one expand the population of endothelial cells in the buffy coat cells? Should it not be to expand the population of endothelial cells from said buffy coat cells?

In claim 8, it is unclear what is encompassed by the phrase "wherein the cell culture medium comprises about 0.5-10 vol-% fetal bovine serum and about 95-99.5 vol-% of a cell culture medium". How can a cell culture medium comprise 10% vol-% fetal bovine serum and about 95% vol-% of a cell culture medium as recited? Clarification is requested since the metes and bounds of the claim can not be clearly determined.

In claim 9, it is unclear what is encompassed by the phrase "resuspended in cell culture medium". It is not clear whether the cell culture medium in a continued culture is the same as that of claim 1 from which claim 9 is dependent upon. Additionally, it is unclear what is encompassed by the phrase "fibronectin/gelatin-coated surface". Does it mean fibronectin or gelatin-coated surface or fibronectin and gelatin coated surface? The metes and bounds of the claim are not clearly determined.

Claim Rejections - 35 USC § 102

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

Claim 44 is rejected under 35 U.S.C. 102(b) as being anticipated by Asahara et al. (Science 275:964-967, 1997) or Levine et al. (U.S. Patent No. 5,132,223).

Asahara et al. teach a proliferated spindle shaped endothelial cell culture population with surface cell markers CD34, CD31, flk-1, Tie-2, E selectin among others prepared from CD34-positive mononuclear blood cells freshly isolated from human peripheral blood (see abstract and page 965, col.1-col. 2). Levine et al. disclose expanded endothelial cell populations derived from human umbilical vein and adult human blood vessels (see Brief summary of the invention). Since the proliferated endothelial cell population of Asahara et al. or that of Levine et al. is indistinguishable from a population of expanded endothelial cells prepared by the method of the instant invention, Asahara et al. and Levine et al. anticipate the instant claim.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and

> the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

Claims 1-2, 5-6, 8-9 and 44 are rejected under 35 U.S.C. 103(a) as being unpatentable over Dzau et al. (U.S. 6,352,555 with an effective filing date of 7/10/1998) in view of Asahara et al. (Science 275:964-967, 1997).

Dzau et al. teach a method for obtaining an endothelial cell culture by obtaining a sample of mononuclear cells taken from a "buffer coat" fraction of a peripheral blood sample (including human sample) and culturing the sample of mononuclear cells on a cell adhesive polymer-coated solid support in the presence of endothelial growth factors (col. 3, lines 6-22; col. 7, lines 12-44; col. 5, lines 19-33), wherein the cell adhesive polymer is any polymer which provides a substrate for endothelial cell attachment including, without limitation, fibronectin, vitronectin, laminin, keratin, gelatin and collagen (col. 3, lines 57-61); the solid support is any solid surface which can support cell growth or differentiation including without limitation a tissue culture plate or well, bead, slide, column, bottle or other vessel (col. 4, lines 1-4); and the endothelial cell growth factors

include VEGF, bFGF, IGF or any combination therefore (col. 3, lines 19-20). Dzau et al. further teach that Endothelial Growth Media supplemented with saturating concentrations of endothelial cell growth factors (VEGF, bFGF and IGF) or the endothelium cell culture medium described by Shi et al. (Blood 92:362-367, 1998) can be used for selective attachment and differentiation of early endothelial progenitor cells (none of the disclosed media contains of bovine brain extract), and that the cultures are expanded *in vitro* for a period of 10-14 days (col. 7, lines 23-44). The cell culture medium described by Shi et al. comprises 10% fetal bovine serum in M199 medium containing VEGF (10 ng/mL), bFGF (1 ng/mL) and IGF (2 ng/mL) (page 363, col. 1, bottom of first paragraph). Drau et al. do not specifically teach that collagen type I to be coated on a solid support such as a tissue culture plate or well. Additionally, Drau et al. do not specifically teach that the cultured cells in the process of claim 1 are trypsinized at about 10^3-fold expansion, collected by centrifuagation, resuspended in cell culture medium, and subjected to continued culture in contact with fibronectin or gelatin-coated surface. However, it is so well known in the art that type I collagen or fibronectin have been used to coat a tissue culture plate or well for an endothelial cell culture. Asahara et al. teach that putative progenitor endothelial cells isolated from human peripheral blood (MB^{CD34+} cells) were cultured on collagen type I coated tissue culture plastic, and a limited number of cells attached, became spindle shaped and proliferated for 4 weeks (see Fig. 1B and page 964, col. 3, first full paragraph). Asahara et al. also teach that a subset of MB^{CD34+} cells plated on fibronectin-coated tissue culture plastic also promptly

attached and became spindle shaped within 3 days, and the number of attaching cells in culture increased with time (see Fig. 1B).

Accordingly, it would have been obvious and within the scope of skill for an ordinary skilled artisan to modify the method taught by Dzau et al. by specifically utilizing collagen type I as a substrate for endothelial cell attachment, growth and differentiation on a solid support as it has been used in a cell culture taught by Asahara et al. One of ordinary skilled artisan would have been motivated to carry out the above modification simply because of a designer's choice, and since it is also so well known in the art that type I collagen can be used to coat a tissue culture plate or well for an endothelial cell culture. With respect to the limitation recited in claim 9, it would also have been obvious and within the scope of skill for an ordinary skilled artisan to trypsinize the cultured cells at about 10^3-fold expansion, collected by centrifugation, resuspended in cell culture medium, and subjected to continued culture in contact with fibronectin or gelatin-coated surface. One of ordinary skilled artisan would have been motivated to carry out this modification in order to further expand the cultured population of endothelial cells, particularly for one already at a high cell density after a 10^3-fold expansion.

Therefore, the claimed invention as a whole was *prima facie* obvious in the absence of evidence to the contrary.

Claims 1 and 3 are rejected under 35 U.S.C. 103(a) as being unpatentable over Dzau et al. (U.S. 6,352,555 with an effective filing date of 7/10/1998) in view of Asahara

et al. (Science 275:964-967, 1997) as applied to claims 1-2, 5-6, 8-9 and 44 above, and further in view of Levine et al. (U.S. Patent No. 5,132,223; IDS).

The combined teachings of Dzau et al. and Asahara et al. have been discussed above. However, none of the references specifically teaches that the cell culture medium comprises heparin, dextran sulfate or mixtures thereof.

However, at the effective filing date of the present application, Levine et al. teach that heparin and/or a dextran sulfate greatly potentiate the stimulatory effect of endothelial cell growth factor on the proliferation of human umbilical vein endothelial cells and of endothelial cells from adult human blood vessels (see the entire patent, particularly Brief summary of the invention).

Accordingly, it would have been obvious for an ordinary skilled artisan at the effective filing date of the present application to modify the modified method resulting from the combined teachings of Dzau et al. and Asahara et al. by further incorporating heparin and/or dextran sulfate into the cell culture medium. One of ordinary skilled artisan would have been motivated to carry out the above modification in order to attain the potentiating effects of heparin and/or dextran sulfate on the stimulatory effect of endothelial cell growth factors, for this instance VEGF, bFGF or IGF as taught by Levine et al. Therefore, the claimed invention as a whole was *prima facie* obvious in the absence of evidence to the contrary.

Claims 1, 4-5 and 7 are rejected under 35 U.S.C. 103(a) as being unpatentable over Dzau et al. (U.S. 6,352,555 with an effective filing date of 7/10/1998) in view of

Asahara et al. (Science 275:964-967, 1997) as applied to claims 1-2, 5-6, 8-9 and 44 above, and further in view of Gupta et al. (Exp. Cell Res. 230:244-251, 1997).

The combined teachings of Dzau et al. and Asahara et al. have been discussed above. However, none of the references specifically teaches that the buffy coat cells are obtained by washing cells from a buffy coat layer obtained from human blood in cell culture medium comprising 20% human male serum or a cell culture medium containing human epidermal growth factor.

At the effective filing date of the present application, Gupta et al. teach that microvascular endothelial cell culture medium comprising MCDB131 endothelial cell culture medium supplemented with VEGF and 20% male human serum (HUS) can be used to culture isolated human dermal microvascular endothelial cells (page 245, col. 1, third full paragraph). Gupta et al. further teach that human dermal microendothelial cells have been cultivated in a culture medium containing 30% human serum and epidermal growth factor as well as in serum-free medium containing epidermal growth factor (page 245, col. 1, fourth full paragraph; page 250, bottom of col. 1).

Accordingly, it would have been obvious and within the scope of skill for an ordinary skilled artisan to modify the method resulting from the combined teachings of Dzau et al. and Asahara et al. by using the cell culture medium containing 20% male human serum (HUS) taught by Gupta et al. to wash buffy coat cells obtained from human blood, and by further adding human epidermal growth factor into the cell culture medium. One of ordinary skilled artisan would have been motivated to carry out the above modifications simply because of the designer's choice because the microvascular

endothelial cell culture medium of Gupta et al. are suitable for culturing endothelial cells as well as the culture media taught by Dzau et al. and Shi et al., and that human epidermal growth factor has been utilized as a component of a culture medium for human microvascular endothelial cells. Therefore, the claimed invention as a whole was *prima facie* obvious in the absence of evidence to the contrary.

Claims 1 and 13-14 are rejected under 35 U.S.C. 103(a) as being unpatentable over Dzau et al. (U.S. 6,352,555 with an effective filing date of 7/10/1998) in view of Asahara et al. (Science 275:964-967, 1997) as applied to claims 1-2, 5-6, 8-9 and 44 above, and further in view of Solovey et al. (NEJM 337:1584-1590, 1997).

The combined teachings of Drau et al. and Asahara et al. have been discussed above. However, none of the references teaches that the expanded population comprises microvascular endothelial cells that are CD34+, CD36+ and expressing the P1H1 antigen. However, Solovey et al. teach that buffy coat cells obtained from human peripheral blood contain circulating endothelial cells expressing CD36 (a distinguishing marker for microvascular endothelial cells), P1H1 antigen and CD34+ (see page 1585, col. 1, under "identification of endothelial cells"; page 1586, col. 2, last paragraph; page 1587, col. 2, last paragraph). Accordingly, it would have been obvious that the expanded population in the endothelial cell culture obtained from a sample of mononuclear cells taken from a "buffer coat" fraction of a peripheral blood sample (including human sample) derived from the modified method of Dzau et al. and Asahara

et al. would comprise microvascular endothelial cells possessing CD34+, CD36+ and P1H1 antigen based on the teachings of Solovey et al.

Therefore, the claimed invention as a whole was *prima facie* obvious in the absence of evidence to the contrary.

Claims 1 and 10-12 are rejected under 35 U.S.C. 103(a) as being unpatentable over Dzau et al. (U.S. 6,352,555 with an effective filing date of 7/10/1998) in view of Asahara et al. (Science 275:964-967, 1997) as applied to claims 1-2, 5-6, 8-9 and 44 above, and further in view of Dementriou et al. (U.S. Patent No. 6,140,123).

The combined teachings of Drau et al. and Asahara et al. have been discussed above. However, none of the references teaches that the cultured cells are subjected to cryopreservation or wherein the cryopreservation medium comprising fetal calf serum containing an effective amount of dimethylsuloxide or that the cryopreserved cells are thawed and culturing is resumed in the cell culture medium.

At the effective filing date of the present application, Demetriou et al. teach that cells have been routinely harvested and preserved in scientific research and development; and when the preserved cells are to be used, they are thawed and placed in a cell culture medium. Demetriou et al. further teach that a wide used cell storage medium is DMEM containing 10 wt. % fetal calf serum and cryopreservatives such as DMSO (see cols. 1-2).

Accordingly, it would have been obvious and within the scope of skill for an ordinary skilled artisan to subject the cultured cells in the modified method resulting

from the combined teachings of Dzau et al. and Asahara et al. to cryopreservation for future uses or for further expansion of the cryopreserved cells in the same culture conditions at any time in the future based on the teachings of Demetriou et al. One of ordinary skilled artisan would have been motivated to carry out the above modification simply depending on a designer's choice.

Therefore, the claimed invention as a whole was *prima facie* obvious in the absence of evidence to the contrary.

Conclusions

No claims are allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Quang Nguyen, Ph.D., whose telephone number is (703) 308-8339.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's mentor, Dave Nguyen, may be reached at (703) 305-2024, or SPE, Irem Yucel, Ph.D., at (703) 305-1998.

Any inquiry of a general nature or relating to the status of this application should be directed to Patent Analyst, Tracey Johnson, whose telephone number is (703) 305-2982.

To aid in correlating any papers for this application, all further correspondence regarding this application should be directed to Group Art Unit 1636.

Quang Nguyen, Ph.D.

DAVE T. NGUYEN
PRIMARY EXAMINER